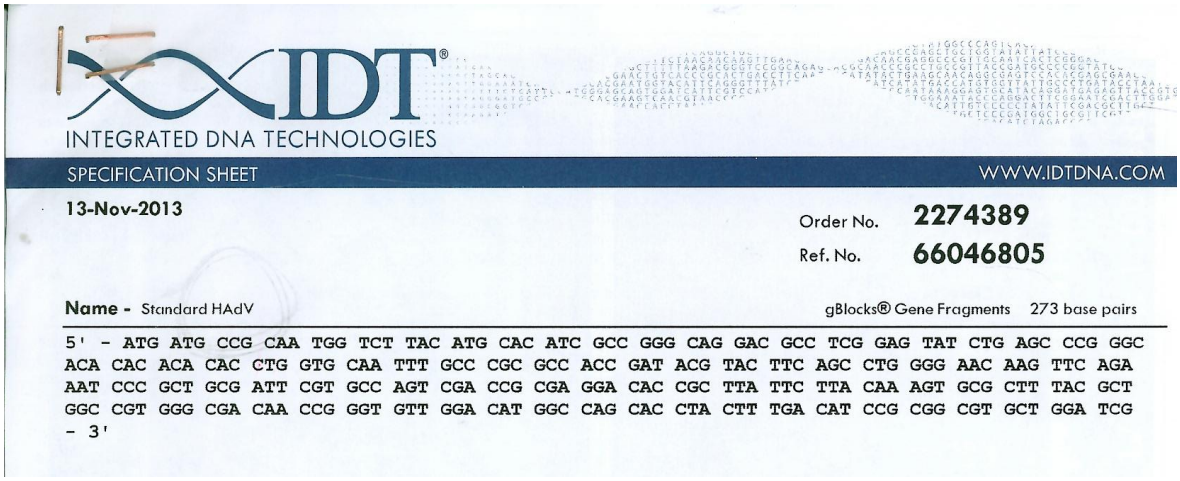
METAWATER

**CONSTRUCTION OF STANDARD CURVES using GBlock**

1. Create an account in www.idtdna.com
2. Go to products and services
3. Select gBlocks® Gene Fragments. The price depends on the size of the DNA sequence
4. Select Order
5. Insert name of the sequence and the length of the synthetic DNA you want.

For HAdV



IDT®
INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET WWW.IDTDNA.COM

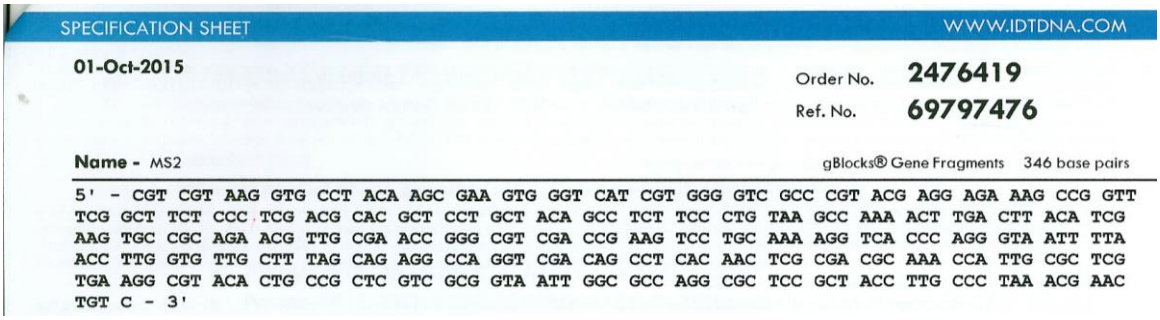
13-Nov-2013

Order No. 2274389
Ref. No. 66046805

**Name -** Standard HAdV gBlocks® Gene Fragments 273 base pairs

5' - ATG ATG CCG CAA TGG TCT TAC ATG CAC ATC GCC GGG CAG GAC GCC TCG GAG TAT CTG AGC CCG GGC
ACA CAC ACA CAC CTG GTG CAA TTT GCC CGC GCC ACC GAT ACG TAC TTC AGC CTG GGG AAC AAG TTC AGA
AAT CCC GCT GCG ATT CGT GCC AGT CGA CCG CGA GGA CAC CGC TTA TTC TTA CAA AGT GCG CTT TAC GCT
GGC CGT GGG CGA CAA CCG GGT GTT GGA CAT GGC CAG CAC CTA CTT TGA CAT CCG CGG CGT GCT GGA TCG
- 3'

For MS2



SPECIFICATION SHEET WWW.IDTDNA.COM

01-Oct-2015

Order No. 2476419
Ref. No. 69797476

**Name -** MS2 gBlocks® Gene Fragments 346 base pairs

5' - CGT CGT AAG GTG CCT ACA AGC GAA GTG GGT CAT CGT GGG GTC GCC CGT ACG AGG AGA AAG CCG GTT
TCG GCT TCT CCC TCG ACG CAC GCT CCT GCT ACA GCC TCT TCC CTG TAA GCC AAA ACT TGA CTT ACA TCG
AAG TGC CGC AGA ACG TTG CGA ACC GGG CGT CGA CCG AAG TCC TGC AAA AGG TCA CCC AGG GTA ATT TTA
ACC TTG GTG TTG CTT TAG CAG AGG CCA GGT CGA CAG CCT CAC AAC TCG CGA CGC AAA CCA TTG CGC TCG
TGA AGG CGT ACA CTG CCG CTC GTC GCG GTA ATT GGC GCC AGG CGC TCC GCT ACC TTG CCC TAA ACG AAC
TGT C - 3'

6. When the stock arrive follow instruction for resuspension
7. Centrifuge the tube for 3-5 sec at a minimum of 3000 x g to ensure the material is in the bottom of the tube.
8. Add TE to reach a final concentration of 10 ng/ul. In the sheet attach with the product you can see the amount of DNA in ng.

METAWATER

| Properties | |
|---|---|
| Length: | 346 |
| Amount Delivered: | 500ng |
| GC Content: | 58.09% |
| Molecular Weight: | 213696.6 |
| fmoles/ng: | 4.68 |
| µg/$OD_{260}$: | 50 |

9. Vortex briefly.
10. Incubate at 50º C for 20 minutes.
11. Briefly vortex and centrifuge.
12. The tube resuspended have to be at -20ºC
13. Quantify the gblock to know the exact amount of DNA per ul. We recommend Qubit R to do that. Normally less than 10 ng/ul.
14. Insert the data in the following excel sheet (double click on the table)

| **Concentrations** | | | | | |
|---|---|---|---|---|---|
| qubit measure | 7,29 | ng/ul | | | |
| molecular weight | 213696,6 | | | | |
| lenght in base pairs | 273 | | | | |
| **Standard calculation** | | | | | |
| | | | | | |
| lenght | 273 | **pb** | | | |
| plasmid concentration | 7,29 | **ng/µl** | | | |
| Molecular weight | 213696,60 | **u.m.a** | | | |
| concentration genomic copies /gr | 2,82E+18 | **cg/gr** | | | |
| ng for 1E+11 genomic copies | 35,49 | **ng** | | | |
| volume that has 1E+11 genomic copies | 4,87 | **µl** | | | |
| volumen of TE buffer | 995,13 | **ul** | | | |
| | | | | | |
| | | | | | |
| stock concentration / ml | 1,00E+11 | 1,00E+10 | 1,00E+09 | 1,00E+08 | 1,00E+07 |
| | | | | | |
| | | | | | |
| genomic copies in 10 ul of reaction | 1,00E+09 | 1,00E+08 | 1,00E+07 | 1,00E+06 | 1,00E+05 |
| genomic copies in 5 ul of reaction | 5,00E+08 | 5,00E+07 | 5,00E+06 | 5,00E+05 | 5,00E+04 |

15. The volume that has 1E+E11 genomic copies is diluted with the complement of TE to get 1ml

16. Serially dilute the DNA in order to obtain dilutions per 10 or 5 µl. See the table in the excel sheet.
17. Test each dilution by triplicate in a qPCR reaction. Make sure signal (fluorescence, Ct values<40) appear after $5x10^0$ copies/5µl for RNA or $1x10^0$ copies/10µl for DNA triplicates. If the signal appears at lower dilutions, the standard curve is not well constructed.

**Annex**

**Sequences of the GBlock for the qPCR of Human adenoviruses and the bacteriophage MS2**

>HAdV

ATGATGCCGCAATGGTCTTACATGCACATCGCCGGGCAGGACGCCTCGGAGTATCTGAGC
CCGGGCACACACACACACCTGGTGCAATTTGCCCGCGCCACCGATACGTACTTCAGCCTG
GGGAACAAGTTCAGAAATCCCGCTGCGATTCGTGCCAGTCGACCGCGAGGACACCGCTTA
TTCTTACAAAGTGCGCTTTACGCTGGCCGTGGGCGACAACCGGGTGTTGGACATGGCCAG
CACCTACTTTGACATCCGCGGCGTGCTGGATCG

>MS2

CGTCGTAAGGTGCCTACAAGCGAAGTGGGTCATCGTGGGGTCGCCCGTACGAGGAGAAA
GCCGGTTTCGGCTTCTCCCTCGACGCACGCTCCTGCTACAGCCTCTTCCCTGTAAGCCAA
AACTTGACTTACATCGAAGTGCCGCAGAACGTTGCGAACCGGGCGTCGACCGAAGTCCTG
CAAAAGGTCACCCAGGGTAATTTTAACCTTGGTGTTGCTTTAGCAGAGGCCAGGTCGACAG
CCTCACAACTCGCGACGCAAACCATTGCGCTCGTGAAGGCGTACACTGCCGCTCGTCGCG
GTAATTGGCGCCAGGCGCTCCGCTACCTTGCCCTAAACGAACTGTC